Prenatal vitamin D3 supplementation: pharmacology and offspring health outcomes

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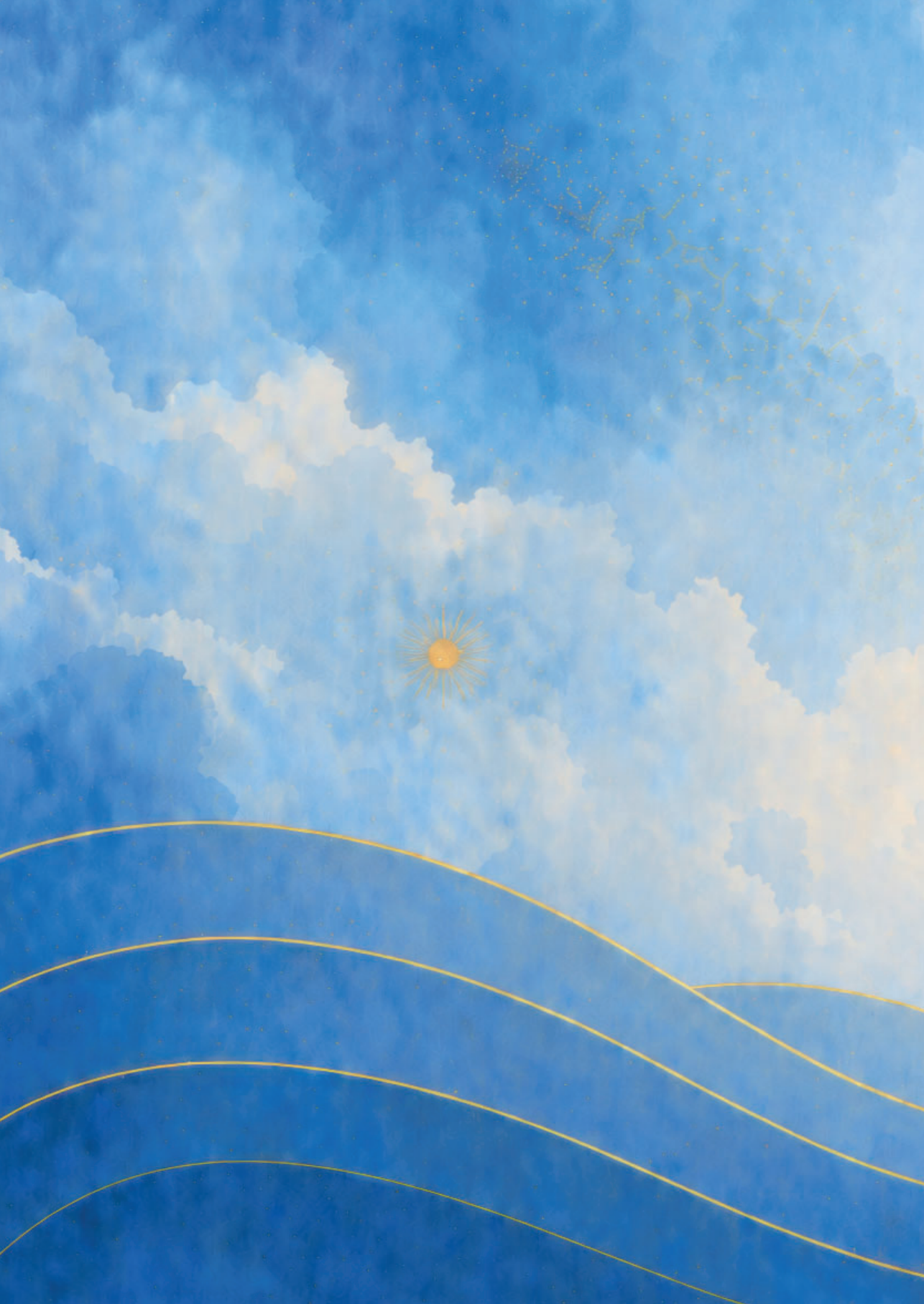

Chapter 1

General introduction

Evolutionary origins and biological significance

Vitamin D, often called the "sunshine molecule", is hypothesized to be one of the oldest evolutionary conserved molecules, having been identified in primitive marine species that emerged between 0.5–1 billion years ago. These species, such as the phytoplankton Emiliania huxlei and jawless primitive fish (Petromyzon marinus), indicate that the vitamin D endocrinology system originated before the development of calcified structures.[1,2] Although its ancient functions are yet to be fully elucidated, researchers have speculated that vitamin D served to protect UV-sensitive macromolecules of early photosynthesizing eukaryotes from radiation damage, as the UV absorption spectra of vitamin D overlap with the high-extinction coefficients of DNA, RNA and key proteins.[1,3]

In humans, vitamin D has traditionally been recognized for its vital function in maintaining calcium and phosphate homeostasis and bone mineralization. However, it has since established itself as a pleiotropic molecule that could affect numerous other physiological functions, including immunomodulation, apoptosis, cellular differentiation, and proliferation across several organ systems.[4,5] Moreover, vitamin D metabolism shows notable adaptations during pregnancy, indicating a profound role in maternal and fetal health that may extend well beyond the perinatal period.[6] These non-calcemic genomic activities of vitamin D are mediated primarily through autocrine and paracrine cell signaling rather than its well-characterized endocrine pathways, opening new fields of vitamin D research at the intersection of immunology, endocrinology and developmental biology.[7] Therefore, while historically recognized for its skeletal functions, as a cure for rickets, vitamin D is experiencing an academic renaissance that highlights our ever-evolving understanding of this ancient molecule.

Vitamin D forms and metabolism

Vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) are the two major forms of vitamin D. Vitamin D_3 is endogenously synthesized in the skin of vertebrates and other animals, as well as found in animal-based foods, whereas vitamin D_2 is produced by ultraviolet-irradiated plants and fungi. In the skin, epidermal 7-dehydrocholesterol undergoes photolysis after exposure to ultraviolet B (UVB) light (280–315 nm) to form pre-vitamin D, which isomerizes rapidly into the secosteroid vitamin D_3.[8] Important variables that modify this process include geographic latitude, time of day, season, age, indoor living, skin pigmentation and UVB-blocking clothing or sunscreen, which can alter cutaneous output.[9]

Micelle production in the presence of bile salts facilitates absorption in the small intestine through passive diffusion of vitamin D, whether it be D_3 or D_2, although simultaneous active transport across the enterocyte membrane via cholesterol transporters adds to the complexity of vitamin D's intestinal absorption.[10] In addition, the fat content of the meal or vitamin D supplement formulation alters absorption, as do interactions with the host microbiome.[11] Vitamin D is then integrated into chylomicrons and other lipoproteins that travel through the lymphatic system to eventually reach systemic circulation.[12]

Once in the bloodstream, vitamin D binds to vitamin D binding protein (DBP), a carrier produced in the liver, which transports it to target sites and, in some tissues, facilitates active uptake.[13] The primary circulating form and current clinical indicator of vitamin D status, 25-hydroxyvitamin D [25(OH)D], is produced when hepatic 25-hydroxylase (CYP2R1) catalyzes hydroxylation of vitamin D in the liver. Due to vitamin D_3 being the endogenously produced form, it is present in higher concentrations than D_2. Moreover, vitamin D_3 is more potent in raising and maintaining 25(OH)D levels because it has higher binding affinity for DBP than D_2, making D_3 the preferred supplementary formula.[14] Therefore, vitamin D_3 is the focus of most vitamin D research, as well as the primary focus of this thesis.

Activation and classical skeletal functions

After delivery to the kidneys, the enzyme 1α-hydroxylase (CYP27B1) metabolizes 25(OH)D into the biologically active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which is the ligand to the vitamin D receptor (VDR), a nuclear transcription factor.[8] Renal 24-hydroxylation (CYP24A1) of 25(OH)D can also occur, generating 24,25-dihydroxyvitamin D [24,25$(OH)_2$D], which possesses little biological activity, and its more polar derivatives are better excreted in the bile and feces. Nevertheless, the role of 24,25$(OH)_2$D, besides regulation of 1,25$(OH)_2$D levels, is gaining renewed interest as a marker of vitamin D status in conjunction with 25(OH)D.[15]

Formation of physiologically active 1,25$(OH)_2$D is thightly controlled by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and serum calcium and phosphate levels, resulting in a greatly decreased half-life of hours, compared to weeks for 25(OH) D. Notably, CYP27B1 is expressed by numerous extra-renal tissues such as the placenta, immune cells, thyroid gland, bladder and eyes, allowing for local synthesis of 1,25$(OH)_2$D to support auto- and paracrine function.[16]

When 1,25$(OH)_2$D enters the nucleus and binds to its nuclear receptor VDR, the complex interacts with the retinoid X receptor to form a heterodimer structure. The heterodimer

engages the vitamin D response elements (VDREs) present in the promotor regions of vitamin D-responsive genes, which numbers are estimated to be in the hundreds, modulating the expression of approximately 1–5% of the human genome.[17]

The classical effects mediated by VDR activation regulate the calcium and phosphate equilibrium, essential for bone health.[18] The upregulated absorption of dietary calcium and phosphate occurs in the intestines, where genomic VDR activity is associated with induction of epithelial calcium channel TRPV6 and calcium binding protein calbindin-D_{9k}. Similarly, sodium-dependent phosphate transporter 2 (PiT-2) and type 2b (NaPi2b) facilitate intestinal absorption of inorganic phosphate. In the kidneys, calcium reabsorption in the distal renal tubule is facilitated by enhanced production of proteins such as the calcium channel TRPV5 and transporter calbindin-D_{28k}, which decrease urinary excretion of calcium.

Additionally, $1,25(OH)_2D$ stimulates osteoblasts to synthesize collagenous and non-collagenous extracellular matrix proteins, including type I collagen, osteopontin and osteocalcin, which are necessary to maintain bone mineral density.[18] Furthermore, $1,25(OH)_2D$ stimulates production of FGF23, and inhibits PTH via a negative feedback loop when vitamin D/calcium intake is sufficient. When calcium concentrations fall, the parathyroid gland secretes PTH, again raising $1,25(OH)_2D$ and driving bone remodeling and resorption by activating osteoblasts and stromal cells to produce receptor activator nuclear factor κB ligand (RANKL). These dynamic feedback loops ensure skeletal integrity and bone health, preventing conditions such as rickets or osteomalacia.

Immunomodulatory and pleiotropic effects

The nearly ubiquitous expression of both CYP27B1 and VDR throughout the body has prompted investigators to study the extraskeletal, or pleiotropic, effects of $1,25(OH)_2D$. As a result, important influences of $1,25(OH)_2D$ have been implicated in the cardiometabolic system, cell cycle and differentiation, and most profoundly, the immune system.[19] Almost all immune cells can activate 25(OH)D into $1,25(OH)_2D$ and express VDR for autocrine and paracrine actions, such as modulation of the immune response, both innate and adaptive, facilitating defenses against microbes and viruses, and dampening inflammation and autoimmune responses.[20,21]

For example, autocrine $1,25(OH)_2D$ signaling has been linked to the complement-mediated shutdown of T helper type-1 (Th1) and type-17 (Th17) cells, switching off pro-inflammatory programs of these $CD4^+$ T cell subsets and suppressing cytokines such as interferon-gamma (IFN-γ), interleukin-2 (IL-2) and IL-17, while promoting IL-10.[22] Furthermore, active vitamin

D has been suggested to modulate T helper type-2 (Th2) populations, resulting in a genomic shift to an increasingly anti-inflammatory Th2 responses, which may provide a functional link to vitamin D's role in allergic phenotypes.[23]

Moreover, $1,25(OH)_2D$ has been associated with suppression of B cell proliferation and class-switch recombination, induction of tolerogenic dendritic cell phenotypes, and decreased expression of major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs).[24-26] Finally, macrophages and monocytes show enhanced antimicrobial activities when incubated with $1,25(OH)_2D$, due to synthesis of cathelicidins, antimicrobial peptides that destabilize bacterial and fungal membranes.[27]

Genome-wide and transcriptomic studies, such as ChIP-seq and RNA-seq, have identified numerous target genes of the VDR-$1,25(OH)_2D$ complex, offering mechanistic insights into its function in immune regulation through direct binding of immune-related VDREs, interactions with transcription factors like NF-κB, and/or epigenetic modifications such as DNA methylation and histone acetylation.[28] The genomic effects of $1,25(OH)_2D$ on equilibrating the immunologic responses suggest potential therapeutic applications in infections, autoimmune and inflammatory diseases.

Vitamin D in pregnancy and fetal development

Pregnancy constitutes a period where the immunomodulatory effects of vitamin D may be particularly significant. A more tolerogenic immune environment is crucial to prevent fetal rejection, as pregnancy naturally represents a controlled inflammatory state, where regulated low-grade inflammation is required for successful implantation of fetal trophoblast into the maternal uterine vasculature and decidua.[29]

During pregnancy, maternal vitamin D metabolism is subject to substantial changes, as evidenced by a pronounced increase in circulatory $1,25(OH)_2D$ concentrations.[30] At no other point in life is vitamin D metabolism altered to such an extent. This elevation is facilitated by marked upregulation of maternal renal CYP27B1, as well as the development of the placenta, which is capable of synthesizing $1,25(OH)_2D$, albeit in small amounts.[6] Despite moderate modifications of serum 25(OH)D, pregnant individuals demonstrate these elevated $1,25(OH)_2D$ levels from early pregnancy, signaling greater maternal and fetal demands and an important role in successful fetal development.

Vitamin D has been suggested to affect embryogenesis, organogenesis, and immunological modulation during gestation.[31,32] In particular, animal models of gestational vitamin D

deficiency or VDR-null models have highlighted a phenotype of impaired fetal pulmonary development. Prenatal vitamin D deficiency results in reduction of the differentiation and total count of pulmonary fibroblasts and alveolar epithelial type 2 cells, decreased synthesis of key pulmonary surfactant proteins, compromised structural integrity of the lung, increased neutrophil infiltration and airway resistance through increased airway smooth muscle mass.[32-35] Vitamin D supplementation during gestation was shown to alleviate these deleterious effects.[36] These activities may be due to direct or indirect modulation by $1,25(OH)_2D$ of genes associated with pulmonary development and function.

Clinical evidence and knowledge gaps

In the clinic, meta-analyses of both prospective and retrospective observational cohorts have linked prenatal vitamin D supplementation to improved early respiratory outcomes, including lower rates of asthma, respiratory tract infections, allergic sensitization, wheezing and improvements in offspring lung function.[37-41] VDR polymorphisms have been associated with a higher frequency of adverse respiratory outcomes.[42,43] The mechanisms underlying these relationships have been proposed to relate to modulation of the offspring airway immune profiles and microbiota compositions, inhibition of airway remodeling, enhanced antimicrobial defenses, and maternal metabolomic alterations involved in the sphingomyelin pathway.[44,45]

However, while preclinical models show profound pulmonary impact of prenatal vitamin D deficiency and observational data has shown associations in a similar direction, randomized controlled trials (RCTs) have yielded mixed results. The two largest trials launched to date to study the phenomenon of prenatal vitamin D deficiency, supplementation and childhood asthma are the Vitamin D Antenatal Asthma Reduction Trial (VDAART) and The Copenhagen Prospective Studies on Asthma in Childhood (COPSAC).[46,47] These mother-child cohorts have provided foundational research, spanning more than 20 years, on the origins, prevention, and natural history of childhood asthma and other respiratory disorders in relation to gestational vitamin D supplementation.

Although a potentially protective effect of sufficient vitamin D levels during gestation on offspring respiratory outcomes was observed in both trials, a definitive cause-and-effect relationship was not established.[48] The VDAART and COPSAC trials indicated a complex and multifactorial relationship between prenatal vitamin D and offspring respiratory outcomes, further complicated by differences in baseline vitamin D levels, administration of standard prenatal vitamins containing small amounts of vitamin D in the control groups, discrepancies in supplementation protocols, and other confounding variables.

These findings highlight critical knowledge gaps in our understanding of vitamin D pharmacology during pregnancy and underscore the need for more detailed exposure-response analyses and revised trial design. Sustaining sufficient vitamin D levels during pregnancy may provide more meaningful benefits for mother and child than previously thought, in particular as pregnant women tend to increase their time spent indoors and standard prenatal supplements fail to result in adequate vitamin D levels in a large percentage of people.[30]

Thesis aims and rationale

Given the compelling preclinical evidence and supportive observational data, yet inconclusive RCT results, there is an urgent need to better understand the pharmacology of prenatal vitamin D supplementation and its relationship to childhood respiratory outcomes. This thesis aims to address critical knowledge gaps by elucidating the exposure-response relationships, identifying factors that influence supplementation efficacy, and exploring mechanisms underlying the maternal-fetal vitamin D axis.

Specifically, this research seeks to: (1) characterize vitamin D biodistribution and metabolism in maternal-fetal tissues, (2) define the exposure-response relationship between maternal vitamin D status and childhood asthma outcomes, (3) identify maternal and environmental variables that modify supplementation success, and (4) explore how adverse pregnancy outcomes may influence offspring health through vitamin D-dependent pathways.

Thesis outline

Chapter 2 discusses the properties of vitamin D, its metabolism, and autocrine, paracrine and endocrine function. To explore the different functions of vitamin D, mediated through its major metabolites in blood but also locally across different tissues, we describe the current preclinical and clinical data on intracellular concentrations after oral supplementation in several species.

Identified knowledge gaps on vitamin D biodistribution, particularly during pregnancy in key target tissues such as the placenta and fetus, are addressed in preclinical models in **Chapter 3**.

We subsequently examine the clinical pharmacology of prenatal vitamin D supplementation in **Chapter 4 and 5**. In these chapters, we interrogate the exposure-response relationship

between vitamin D metabolite exposure and childhood asthma and lung function based on the two largest trials on the subject to date, the VDAART and COPSAC trials (**Chapter 4**). We identify variables modifying the efficacy of prenatal vitamin D supplementation to restore maternal circulatory reserves and its association with offspring outcomes (**Chapter 5**).

We further explore the link between adverse pregnancy outcomes or maternal characteristics and offspring outcomes through epidemiological analyses, identifying how several factors during gestation may have long-term impact on children. We describe how the risk of childhood asthma is modified differently in preterm and term neonates by early life weight status (**Chapter 6**) and how maternal weight and gestational weight gain relate to offspring's early life weight (**Chapter 7**). Furthermore, we discuss how adverse pregnancy outcomes, such as preeclampsia, can affect offspring through epigenetic programming at the maternal-fetal interface (**Chapter 8**). We also explore how spontaneous preterm birth and preeclampsia shape the early life infant gut microbiome (**Chapter 9**) and lastly how perinatal factors relate to markers of immuno-inflammation during pregnancy (**Chapter 10**).

Finally, we discuss the current and future implications of our research for optimizing not only prenatal vitamin D supplementation, but also clinical trial design, in the pursuit of improved pregnancy outcomes for women and respiratory health for children in **Chapter 11**.

References

1. Eliason, O., et al., The photo-protective role of vitamin D in the microalga Emiliania huxleyi. Iscience, 2024. 27(6).
2. Whitfield, G.K., et al., Cloning of a functional vitamin D receptor from the lamprey (Petromyzon marinus), an ancient vertebrate lacking a calcified skeleton and teeth. Endocrinology, 2003. 144(6): p. 2704-2716.
3. Holick, M.F., The One-Hundred-Year Anniversary of the discovery of the Sunshine Vitamin D3: Historical, personal experience and evidence-based perspectives. Nutrients, 2023. 15(3): p. 593.
4. Kamen, D.L. and V. Tangpricha, Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. Journal of molecular medicine, 2010. 88: p. 441-450.
5. Samuel, S. and M.D. Sitrin, Vitamin D's role in cell proliferation and differentiation. Nutrition reviews, 2008. 66(suppl_2): p. S116-S124.
6. Hollis, B.W. and C.L. Wagner, New insights into the vitamin D requirements during pregnancy. Bone research, 2017. 5(1): p. 1-16.
7. Morris, H.A. and P.H. Anderson, Autocrine and paracrine actions of vitamin D. The Clinical Biochemist Reviews, 2010. 31(4): p. 129.
8. Bikle, D.D., Vitamin D metabolism, mechanism of action, and clinical applications. Chemistry & biology, 2014. 21(3): p. 319-329.
9. Wacker, M. and M.F. Holick, Sunlight and Vitamin D: A global perspective for health. Dermato-endocrinology, 2013. 5(1): p. 51-108.
10. Reboul, E., Intestinal absorption of vitamin D: from the meal to the enterocyte. Food & function, 2015. 6(2): p. 356-362.
11. Reboul, E., Proteins involved in fat-soluble vitamin and carotenoid transport across the intestinal cells: New insights from the past decade. Progress in Lipid Research, 2023. 89: p. 101208.
12. Blomhoff, R., et al., Lymphatic absorption and transport of retinol and vitamin D-3 from rat intestine evidence for different pathways. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1984. 772(2): p. 109-116.
13. Bouillon, R., et al., Vitamin D binding protein: a historic overview. Frontiers in endocrinology, 2020. 10: p. 910.
14. Tripkovic, L., et al., Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. The American journal of clinical nutrition, 2012. 95(6): p. 1357-1364.
15. Dugar, A., et al., The vitamin D metabolite ratio (VMR) is a biomarker of vitamin D status that is not affected by acute changes in vitamin D binding protein. Clinical Chemistry, 2023. 69(7): p. 718-723.
16. Bikle, D.D., Vitamin D: Newer concepts of its metabolism and function at the basic and clinical level. Journal of the Endocrine Society, 2020. 4(2): p. bvz038.
17. Carlberg, C., Vitamin D and its target genes. Nutrients, 2022. 14(7): p. 1354.
18. Dusso, A.S., A.J. Brown, and E. Slatopolsky, Vitamin D. Am J Physiol Renal Physiol, 2005. 289(1): p. F8-28.
19. Bikle, D.D., S. Patzek, and Y. Wang, Physiologic and pathophysiologic roles of extra renal CYP27b1: Case report and review. Bone reports, 2018. 8: p. 255-267.
20. Gayan Ramirez, G. and W. Janssens, Vitamin D actions: the lung is a major target for vitamin D, FGF23, and Klotho. Journal of Bone and Mineral Research Plus, 2021. 5(12): p. e10569.
21. Hewison, M., Vitamin D and immune function: autocrine, paracrine or endocrine? Scandinavian Journal of Clinical and Laboratory Investigation, 2012. 72(sup243): p. 92-102.
22. Chauss, D., et al., Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells. Nature immunology, 2022. 23(1): p. 62-74.
23. Kilic, A., et al., Vitamin D constrains inflammation by modulating the expression of key genes on Chr17q12-21.1. Elife, 2024. 12: p. RP89270.
24. Chen, S., et al., Modulatory effects of 1, 25-dihydroxyvitamin D3 on human B cell differentiation. The Journal of Immunology, 2007. 179(3): p. 1634-1647.

25. Ferreira, G.B., et al., Vitamin D3 induces tolerance in human dendritic cells by activation of intracellular metabolic pathways. Cell reports, 2015. 10(5): p. 711-725.
26. Vanherwegen, A.-S., C. Gysemans, and C. Mathieu, Vitamin D endocrinology on the cross-road between immunity and metabolism. Molecular and cellular endocrinology, 2017. 453: p. 52-67.
27. Liu, P.T., et al., Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science, 2006. 311(5768): p. 1770-1773.
28. Koivisto, O., A. Hanel, and C. Carlberg, Key vitamin D target genes with functions in the immune system. Nutrients, 2020. 12(4): p. 1140.
29. Bulla, R., F. Bossi, and F. Tedesco, The complement system at the embryo implantation site: friend or foe? Frontiers in immunology, 2012. 3: p. 55.
30. Hollis, B.W., et al., Vitamin D supplementation during pregnancy: Double-blind, randomized clinical trial of safety and effectiveness. Journal of bone and mineral research, 2011. 26(10): p. 2341-2357.
31. Tamblyn, J., et al., Immunological role of vitamin D at the maternal–fetal interface. Journal of Endocrinology, 2015. 224(3): p. R107-R121.
32. Wagner, C.L. and B.W. Hollis, The implications of vitamin D status during pregnancy on mother and her developing child. Frontiers in endocrinology, 2018. 9: p. 500.
33. Foong, R.E., et al., The effects of in utero vitamin D deficiency on airway smooth muscle mass and lung function. American journal of respiratory cell and molecular biology, 2015. 53(5): p. 664-675.
34. Zosky, G.R., et al., Vitamin D deficiency causes deficits in lung function and alters lung structure. American journal of respiratory and critical care medicine, 2011. 183(10): p. 1336-1343.
35. Chen, L., et al., Identification of vitamin D sensitive pathways during lung development. Respiratory research, 2016. 17: p. 1-11.
36. Yurt, M., et al., Vitamin D supplementation blocks pulmonary structural and functional changes in a rat model of perinatal vitamin D deficiency. American Journal of Physiology-Lung Cellular and Molecular Physiology, 2014. 307(11): p. L859-L867.
37. Zosky, G.R., et al., Vitamin D deficiency at 16 to 20 weeks' gestation is associated with impaired lung function and asthma at 6 years of age. Annals of the American Thoracic Society, 2014. 11(4): p. 571-577.
38. Knihtilä, H.M., et al., Low gestational vitamin D level and childhood asthma are related to impaired lung function in high-risk children. Journal of Allergy and Clinical Immunology, 2021. 148(1): p. 110-119. e9.
39. Loddo, F., et al., Association of maternal gestational vitamin D supplementation with respiratory health of young children. Nutrients, 2023. 15(10): p. 2380.
40. Allan, K.M., et al., Maternal vitamin D and E intakes during pregnancy are associated with asthma in children. European Respiratory Journal, 2015. 45(4): p. 1027-1036.
41. Pacheco-González, R.M., L. García-Marcos, and E. Morales, Prenatal vitamin D status and respiratory and allergic outcomes in childhood: a meta-analysis of observational studies. Pediatric Allergy and Immunology, 2018. 29(3): p. 243-253.
42. Roth, D.E., et al., Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. The Journal of infectious diseases, 2008. 197(5): p. 676-680.
43. Ashok, N., R. Kirubakaran, and R. Saraswathy, Association of vitamin D gene polymorphisms in children with asthma-A systematic review. Heliyon, 2020. 6(9).
44. Brustad, N. and B. Chawes, Vitamin D primary prevention of respiratory infections and asthma in early childhood: evidence and mechanisms. The Journal of Allergy and Clinical Immunology: In Practice, 2024. 12(7): p. 1707-1714.
45. Hall, S.C., K.D. Fischer, and D.K. Agrawal, The impact of vitamin D on asthmatic human airway smooth muscle. Expert review of respiratory medicine, 2016. 10(2): p. 127-135.
46. Litonjua, A.A., et al., The Vitamin D Antenatal Asthma Reduction Trial (VDAART): rationale, design, and methods of a randomized, controlled trial of vitamin D supplementation in pregnancy for the primary prevention of asthma and allergies in children. Contemp Clin Trials, 2014. 38(1): p. 37-50.
47. Bisgaard, H., et al., Deep phenotyping of the unselected COPSAC2010 birth cohort study. Clin Exp Allergy, 2013. 43(12): p. 1384-94.

48. Wolsk, H.M., et al., Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. PLoS One, 2017. 12(10): p. e0186657.